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THE INFLUENCE OF PUPAL AGE ON SENSITIVITY TO RADIATION [1]

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In recent years there has been increasing interest in the application of ionizing radiations in the control of insects, and increasing recognition of the usefulness of insects as experimental subjects in the investigation of radiobiological phenomena. For some types of radiobiological study, the germicidal wave-lengths of ultraviolet (UV) radiation, since they are preferentially absorbed by nucleic acids, may be employed more advantageously than the ionizing wave-lengths of x- and gamma radiations. Furthermore, sources of UV radiation are inexpensive, relatively non-hazardous, and readily available. Unfortunately, interpretation of the effects of UV on metazoa may be complicated by the fact that the radiation penetrates only a few cell layers; this explains the paucity of data on UV effects on insects, except for those on insect eggs.

As part of a more extensive investigation of recovery mechanisms in irradiated *Tribolium* (Ducoff and Walburg, 1960; Ducoff and Bosma, 1963) we have compared the effects of x-rays and germicidal UV on pupal stages of *T. confusum*. The two types of radiation differed markedly in regard to stage of greatest sensitivity and to the relative importance of lethal effects and induction of developmental abnormalities.

Materials and Methods

The *T. confusum* stock employed was derived from the colony of G. Fraenkel; the beetles were maintained in white flour supplemented (4%) with brewer's yeast, at 30° C. and 70% R. H. Individual cultures were kept in previously washed polystyrene vials fitted with perforated snap-caps. X-irradiation was performed with a Picker Vanguard deep-therapy machine, operated at 280 kvp and 20 ma; HVL was 1.0 mm. copper, and subject-target distance was 40 cm., yielding a dose rate of approximately 250 r/min., as measured with a Victoreen condenser meter. The UV source was a 15-watt germicidal lamp in a desk-type fluorescent fixture; this gave intensities of about 250 ergs/mm.^{2}/min. at a distance of 100 cm. The output of this type of lamp declines with use, so actual dose rate was measured at the end of each exposure series, using a General Electric Ultraviolet Intensity meter. Care was taken to avoid photoreactivation.

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Homogeneous populations of pupae of known age were obtained in the following manner: Young adult males and females in approximately equal numbers were placed in a large vial of fresh medium for a week. The adults were then removed, and the culture incubated undisturbed for an additional two weeks. The culture was then sifted daily, and those animals which had pupated in the preceding 24 hours removed, sexed, and placed in separate vials of fresh medium.

For irradiation, pupae were freed of medium and distributed in plastic petri dishes, which were uncovered during the exposures.

Results

Preliminary experiments were performed with insects exposed to UV two to four days after pupation. Even doses in excess of 12,000 ergs/mm.[2] did not

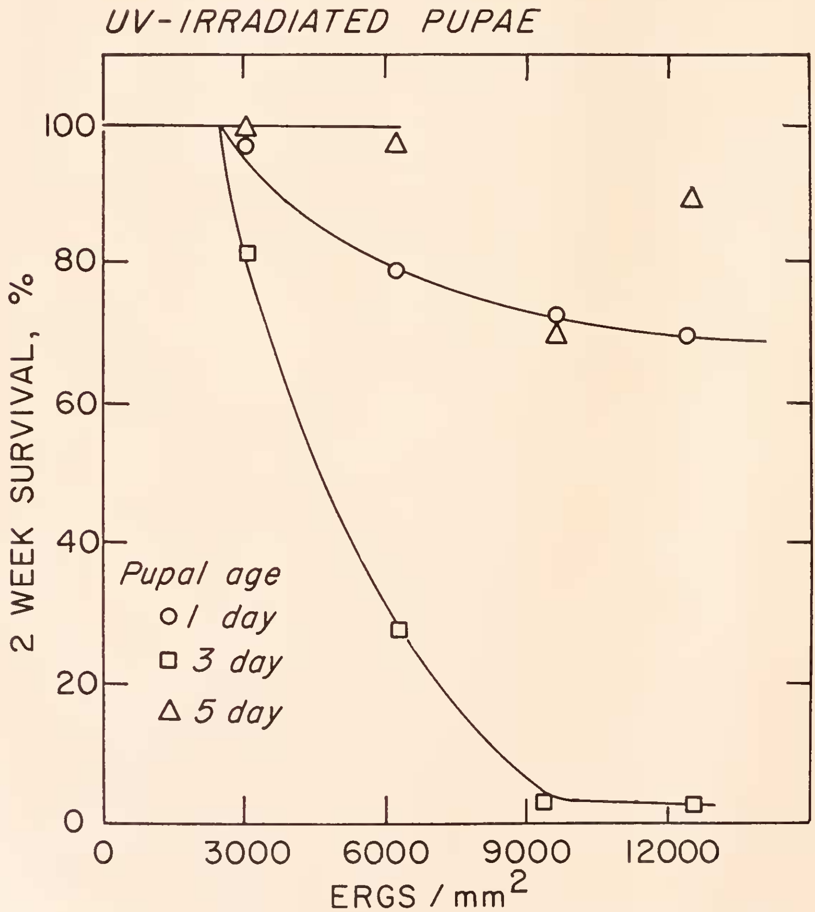

Figure 1. The per cent of *T. confusum* surviving two weeks after exposure, as pupae, to germicidal ultraviolet.

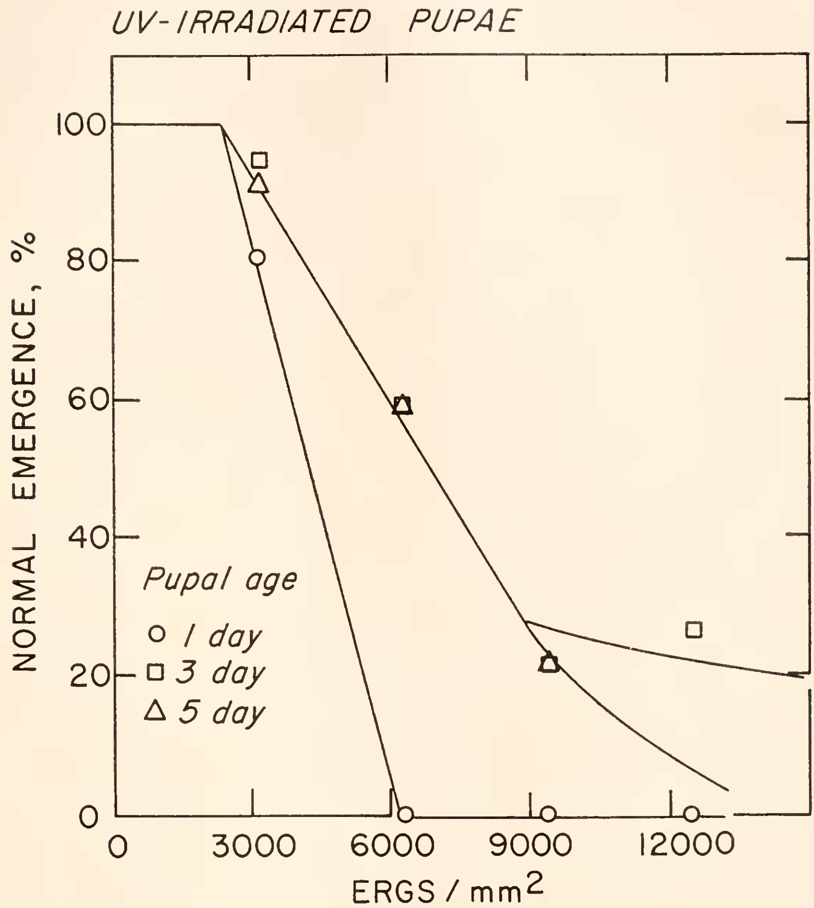

FIGURE 2. The per cent of UV-irradiated *T. confusum* pupae which developed into adults that appeared normal to the unaided eye.

suppress eclosion, but all of the adults exhibited severe wing deformities, and most died during the following week.

When pupae of selected ages were UV-irradiated, there were marked differences in sensitivity. UV radiation was much more effective when administered on the third day of pupation than when administered on either the first or the fifth day, when the parameter scored was survival for two or more weeks after exposure (Fig. 1). On the other hand, if the incidence of emergence without obvious deformities was considered (Fig. 2), the youngest pupae were the most sensitive. Surprisingly, although the abnormalities produced by UV-treatment of one-day and of five-day pupae in these and in similar experiments often appeared severe, they were not necessarily incompatible with life; many adults with gross abnormalities, primarily of the elytra, survived for months after eclosion. By contrast, a number of those UV-irradiated on the third day of pupation gave rise to adults which appeared grossly normal, but which died within a few days.

The sensitivity of three-day-old pupae to the lethal effects of UV was also surprising, in view of reports that *Tribolium* (Beck, 1963; Ducoff and Bosma, 1963) as well as many other insects—*e.g., Drosophila* (Villee, 1946)—show a progressive decline in x-ray sensitivity as pupal development proceeds. Accordingly, a more extensive experiment was performed. Newly-pupated beetles were collected on each of 5 successive days; on the fifth day, sub-groups were exposed to appropriate

TABLE I

X-irradiated pupae

Pupal age (days)	% Survival, 11 days		% Deformed	
	3 kr	5 kr	3 kr	5 kr
1	95	20	95	100
2	75	75	25	80
	9 kr	13 kr	9 kr	13 kr
3	75	65	50	43
4	95	90	9	10
5	100	100	0	0

doses of x- or UV-radiation. The results of the x-irradiation are presented in Table I and those of the UV-irradiation in Table II. These data confirm the previous reports that x-ray resistance increases as pupae develop, with a very abrupt increase about the second day; furthermore, resistance to the production of deformities by x-irradiation parallels resistance to lethal effects. The data also demonstrate that resistance to UV-lethality decreases to a minimum on the third

TABLE II

UV-irradiated pupae

Pupal age (days)	% Survival, 11 days		% Deformed among survivors	
	4200 ergs/mm.^{2}	6500 ergs/mm.^{2}	4200 ergs/mm.^{2}	6500 ergs/mm.^{2}
1	85	55	90	90
2	80	40	100	100
3	30	5	80	100
4	89	80	20	80
5	90	90	30	80

day, and that doses which produce negligible lethality in older pupae nevertheless produce a very high incidence of developmental abnormalities.

DISCUSSION

Sub-lethal doses of UV-radiation proved to be much more efficient in producing developmental abnormalities than sub-lethal or mid-lethal doses of x-radiation.

This result was not unexpected. It seems likely that damage to deeper tissues is necessary to cause death, and that very high doses of the poorly penetrating UV wave-lengths may produce extensive damage to surface structures without producing significant effects in the deeper tissues. In accordance with this, the x-ray effect is highly specific, producing deformities only in the elytra and in the posterior wings (Beck, 1963) ; thus, these deformities appear to be phenocopies of the mutant "split" (McDonald, private communication). Most of the readily-observable UV-induced deformities also involved the wings, but the wing deformities after UV-irradiation were much more varied both in type and in severity, and deformities in other parts of the body were not infrequent.

The spectacular increase in UV-sensitivity on the third day of pupation is more difficult to explain. There may be particularly sensitive cellular processes occurring, or morphogenetic movements may bring sensitive structures close to the surface at that time. Alternatively, the concentration of nucleotides or other UV-absorbing materials might be reduced on the third day. The data of Chaudhary and Lemonde (1963) and of Devi *et al.* (1963) constitute evidence against this possibility, however; these workers found a slightly lowered nucleotide concentration on the first or second day of pupation, and a marked and progressive increase of 260 mμ-absorbing material from prepupal to early adult stages.

The time of maximum sensitivity of *Drosophila* pupae also was different for x-rays (Villee, 1946) than for UV-light (Villee, 1947), and for lethality as compared to phenocopy production by UV. In particular, the flies were more sensitive to killing and more resistant to phenocopy-induction an hour or two after pupation than at any other time during pupal or late larval development. Unfortunately, Villee's interesting UV experiments were performed prior to the recognition of the phenomenon of photoreactivation (Kelner, 1949) and presumably, no precautions were taken to avoid light exposure. Subsequently, Perlitsh and Kelner (1953) demonstrated photoreactivation of phenocopy-production after UV-irradiation of newly-pupated *D. melanogaster.* Thus it is not clear whether the changes in apparent UV-sensitivity in Villee's experiments truly represent changes in sensitivity, or reflect changes in photoreactivating efficiency. It is clear, however, that in *Drosophila,* as in *Tribolium,* the time of maximum sensitivity to the lethal effect of UV does not coincide with the time of maximum sensitivity to UV-induction of developmental abnormalities.

Summary

T. confusum exposed to germicidal UV at any time during the 5-day pupal period exhibits a high incidence of gross morphological deformities, particularly of the wings, in the emerging adult, although the greatest sensitivity to this effect occurs on the first day of pupation. Mortality within two weeks is by far the greatest, however, if UV-irradiation is performed on the third day of pupation. By contrast, sensitivity to both lethality and abnormality-induction after x-ray exposure is greatest on the first day of pupation and declines progressively thereafter, with the sharpest decline occurring late on the second day. These differences are partially explained by the fact that energy absorbed from x-rays is distributed rather uniformly throughout the pupa, whereas most of the UV energy is absorbed in the outermost tissue layers.

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